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Examining the association between blood manganese and lead levels in schoolchildren in four selected regions of South Africa ☆

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Abstract

This study assessed the possibility of relationship between manganese and lead levels in blood in schoolchildren residing in different geographical regions of South Africa. A cross-sectional survey was carried out in schools of three cities [Cape Town (11 schools), Johannesburg (10 schools), Kimberley (six schools) and in the Northern Cape in three rural sites (four schools). A total of 1282 venous blood samples were collected from grade one children. The relationships between blood manganese and blood lead levels (treating each in turn as the response variable in order to adjust for the effect of confounding variables) were investigated by fitting mixed models. Mixed models were fitted with natural log (manganese concentration) as the response variable, and blood lead level as the principal explanatory factor. The model also included terms for centre and a centre by lead interaction and examined potential confounders. The important confounders were found to be gender, race and whether there was paint peeling from the outside walls. There was a significant centre by lead interaction ($P<0.0001$) with the effect of lead on ln(Mn) being different in the various centres. Mixed models fitted with blood lead level as the response variable and blood manganese as the principal explanatory factor, with terms for centre, a centre by manganese interaction and potential confounders, again found overwhelming evidence ($P<0.0001$) of a centre by manganese interaction. The study found great variability in both blood lead and manganese levels, both within and between sites, and there was not a consistent relationship between the two metals in the four sites.

Keywords: Blood lead and manganese; Children; MMT; South Africa

1. Introduction

Environmental sources of known toxic metals are either naturally occurring or of anthropogenic origin emanating, mainly from industrial, agricultural and vehicular emissions. Motor vehicles are an ubiquitous source of airborne emissions of metals that contribute to environmental contamination and are thought to have direct and indirect impact on human health (Gulson et al., 2006). Of particular concern are chronic low-level exposures to toxic metals that can affect health, especially the health of susceptible populations such as pregnant women, developing foetuses, young children, the aged, individuals with communicable diseases such as TB and HIV/AIDS and/or those affected by chronic illness. Populations living in poor socioeconomic conditions that are prevalent in developing countries are also at greater risk.

Vehicular emissions of metals such as lead and manganese, used as octane enhancers, are of particular concern and interest to this study. To date, the use of lead containing petrol additives in the form of tetraethyl lead (TEL) has been phased out in most developed countries or is in the process of being phased out in others. Manganese containing the methylcyclopentadienyl manganese tricar-

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bonyl (MMT) compound, a relatively cheap form of additive, has been used to replace lead in petrol. MMT is manufactured by the Ethyl Corporation (also producer of lead containing TEL) and at present, marketed in many countries, including South Africa. Products of MMT, when emitted as particulate matter from the vehicular exhaust, are converted into inorganic forms of manganese and readily dispersed into environment (Gulson et al., 2006). The environmental fate of these manganese containing compounds is not fully understood, and scientific evidence is needed to prove that they do not pose health risks (US EPA, 1994; Davis, 1998).

Although both lead and manganese are known neurotoxins, able to cross the blood-brain barrier and accumulate in the brain and other target organs, to date most of the research has focused mainly on lead toxicity. A number of studies have reported that the removal of lead from petrol has shown a reduction in exposure levels and lowering of mean blood levels in children (Thomas et al., 1999; Von Storch et al., 2003). Similarly, in South Africa, a longitudinal study that measured blood lead levels changes over time in urban children showed that the reduction of lead content in the country's petrol resulted in the slow decline of blood lead levels overall but concern remains as a large proportion of children still exceed a concentration of 10 μg/dL lead in their blood (17% overall in this study) (Mathee et al., 2006).

The MMT compound is a neurotoxin that is proven to cause agitation and convulsions, as well as pulmonary damage at elevated concentrations to exposed individuals but little is known about the effect of manganese containing vehicle emission products of MMT on health outcomes in community settings (Baldwin et al., 1999).

Such studies are difficult to perform because manganese, unlike lead that is exclusively toxic, is also an essential element in human nutrition needed for cellular metabolic mechanisms and for protection against lipid peroxidation (Wedler, 1994). Furthermore, both clinical deficiency of manganese, although rare, and excess of manganese are associated with specific pathologies. Deficiency of manganese may result in bone and connective tissue defects (Hall et al., 1989), convulsive disorders (Dupont and Tanaka, 1985) and susceptibility to epilepsy (Papavasiliou and Miller, 1983). Excessive intake of manganese on the other hand, both through inhalation or ingestion, may result in pathology, particularly to the central nervous system (CNS) (Mergler and Baldwin, 1997).

A number of studies have investigated the mechanism of manganese uptake and excretion (Crossgrove et al., 2003) and the possibility of synergetic toxic effects of lead and manganese in populations exposed to vehicular emissions. A comparative study of manganese and lead levels in umbilical cord and maternal blood in two large cities (Montreal and Paris) stratified by different gasoline additives were recently reported (Smargiassi et al., 2002). Similarly a high correlation between lead and manganese in human blood was found with an inverse relation to haemoglobin levels in populations residing in different areas of Central Mexico (Santos-Burgoa et al., 2001). Recently, Gulson et al. (2006) reported on changes over time in manganese and lead levels in environment and children, associated with the introduction of MMT in Australian petrol. In occupational settings the effect of lead exposure on the levels of manganese in blood was also reported (Baldwin et al., 1999).

In South Africa, anthropogenic sources of lead and manganese derive primarily from industrial activities and vehicular emissions of petrol additives. At present, in South Africa, petrol producing oil companies are developing alternative formulations as part of ongoing automobile technology development, as well as in preparation for phasing out of lead as an additive in the country's fuels at the beginning of the 2006. To the best of our knowledge, as from 2006, unleaded fuels in South Africa will be either metal free or contain metal additives such as potassium, phosphorous or manganese.

Phase one of our study conducted in Johannesburg and in Cape Town in 2002 showed that 12.5% of children in Johannesburg and 4.2% in Cape Town had blood manganese level equalled or exceeding 14 μg/L, the upper normal values as specified by Agency for Toxic Substances Disease Registry (Röllin et al, 2005; ATSDR, 2000). It is important to state that MMT was added to unleaded petrol in the Johannesburg area 24 months before commencement of the study. The survey continued in other geographical regions of South Africa, namely Northern Cape that included the city of Kimberley and four smaller towns, and found that 8% of children residing in the city of Kimberley also exceeded this limit. The study also examined levels of lead in blood in the same cohort of children.

Since little is known about possible detrimental health effects of concurrent environmental exposures to vehicular emission products of lead and manganese this paper examines the association between manganese and lead blood levels in schoolchildren in four selected regions of South Africa.

2. Materials and methods

A cross-sectional survey was carried out in the cities of Cape Town (11 schools), Johannesburg (10 schools) and in the Northern Cape Province comprising of city of Kimberley (six schools) and three rural sites (four schools). Schools were the primary unit of sampling, and were selected from areas expected to have varying levels of exposure. The total number of venous blood samples collected in three centres was 1282 and each analysed for manganese and lead content. Environmental samples of school playground soil, classroom dust, paint and water were also collected and analysed for manganese and lead content. A questionnaire was also completed by the caregiver of each child in order to obtain information on sociodemographic variables and other potential risk factors. The details of study design, sample collection and methodology are described elsewhere (Röllin et al., 2005).

2.1. Statistical analysis

The relationships between blood manganese level and blood lead level (treating each in turn as the response variable in order to adjust for the

effect of confounding variables) were investigated by fitting linear mixed models. This technique is appropriate to take into account the fact that in the study design pupils were clustered within schools (Snijders and Bosker, 1999; Murray, 1998). In this analysis the schools were considered as being part of four centres namely Cape Town, Johannesburg, Kimberley and rural Orange River Valley (Aggeneys, Pella and Onseepkans). The latter three towns were joined into one centre due to the fact that there were only four schools in these three towns. An examination of the residuals from the fitted models suggested that the blood manganese level should be subject to a log transformation, while the blood lead level did not need to be transformed. We analysed the natural Log of Mn—hereafter denoted as ln(Mn). All analyses were carried out using Stata release 8 (StataCorp, 2003).

3. Results

The study cohort was divided into four study sites namely the three cities of Cape Town, Johannesburg and Kimberley, and in the Northern Cape three rural sites (Pella, Onseepkans and Aggeneys). There were results on both blood level and blood manganese level for a total of 1282 children (the results for one child who had repeated blood lead levels above 40 μg/dL were omitted as it was found that the child regularly ate large quantities of paint from the walls of her house). The results for the blood manganese and lead levels are summarised in Table 1(a) and (b). Table 2(a) and (b) shows manganese and lead concentration in environmental samples collected.

Mixed models were then fitted with the natural log of the manganese blood concentration as the response variable, the blood lead level as the principal explanatory variable, a factor for centre (grouped as Cape Town, Johannesburg, Kimberley and Orange River Valley), potential confounders as explanatory variables and with school as a random effect; thus in the terminology of multilevel models, these are two-level models with the levels being schools and children within schools (Snijders and Bosker, 1999). The potential confounders considered included the variables found to be associated with elevated blood manganese levels (Röllin et al., 2005) and were gender, race (coded as African Black, coloured or other), whether there was paint peeling from the house indoors, whether there was paint peeling from the house outdoors, whether the child exhibited any pica, in particular whether the child ate any paint, whether or not the child had attended a creche before attending school, whether anyone in the house smoked, whether anyone in the house was involved in spray painting, whether the caregiver regarded the house as being very dusty, the father's education level and whether or not there was a lead mine (in order to differentiate the lead mining town from the two rural sites in the Northern Cape). Terms were included both for blood lead level and lead level squared in order to allow for a non-linear relationship between ln(Mn) and blood lead concentration. The results from this modelling are shown in Table 3(a); thus the important confounders were found to be gender (with blood manganese levels being higher for females), race (with manganese levels being lower for Black Africans than for other races) and paint peeling outdoors (which lead to lower manganese levels). Adjusting for these confounders there was evidence of a linear trend of ln(Mn) with increasing blood lead and some evidence of a quadratic effect. A test was then carried out to see whether the quadratic and linear effects could be regarded as being homogeneous over the four centres. There was overwhelming evidence that neither the linear nor the quadratic effects were in fact homogeneous ($P<0.0001$—see Table 3(b)).

To examine this further separate models were fitted to each centre with the following results: Cape Town—a significant linear trend with a slope of 0.025 (increase in ln(Mn) for a unit increase in blood lead level) and no evidence of a quadratic effect; Johannesburg—there was no evidence of a quadratic effect and the linear effect of 0.0016 (less than one-tenth of the Cape Town effect) was not significant; Kimberley showed both a linear and a

Table 1
Concentrations of blood manganese (μg/L) and lead (μg/dL) by study area

Statistic	Cape Town	Johannesburg	Kimberley	Aggeneys	Pella	Onseepkans
(a) *Manganese in the blood of study subjects (μg/L)*						
n	427	381	355	21	55	43
mean	6.75	9.76	9.72	9.86	8.30	7.75
Std dev	3.47	3.53	3.12	2.63	2.74	2.02
Median	6.2	9.2	9.5	9.2	8	7.4
IQR	4.7–7.8	7.4–11.4	7.5–11.2	7.6–11.9	6.5–10.2	6.0–9.5
Range	1.6–32.8	3.6–26.5	1.5–23.7	6.5–16.1	3.3–17.4	3.8–12.2
(b) *Lead in the blood of study subjects (μg/dL)*						
n	427	381	355	21	55	43
Mean	6.44	9.06	7.07	7.76	5.73	5.71
Std dev	2.90	3.11	2.72	2.88	2.58	1.99
Median	6.1	8.9	6.7	7.9	5.1	5.7
IQR	4.5–8.1	6.7–11.3	5.1–8.4	6.0–9.3	4.0–6.6	4.2–7.4
Range	1.0–24.5	1.1–18.1	2.1–22.6	2.8–13.4	2.5–17.1	2.4–12.1
Proportion with Pb $\geqslant$ 10 μg/dL	10.1%	34.7%	9.6%	14.3%	9.1%	2.3%

Std dev, standard deviation; IQR, inter-quartile range.

Table 2
Manganese and lead in the environment of the schools

Statistic	Cape Town	Johannesburg	Kimberley	Aggeneys	Pella	Onseepkans
(a) *Manganese in the environment of the schools*						
n (schools)	11	10	4	2	1	1
Water μg/L						
Range	1.0–15.7	0–3.1	0–1.8	0–0.5	2.7	1.1
Median (IQR)	1.9 (1.3–2.4)	0.6 (0–0.9)	0.6 (0.2–1.3)	n/a	n/a	n/a
Soil (μg/g)						
Range	10–115	117–904	63–240	136–188	144	44
Median (IQR)	18 (13–33)	436 (178–731)	137 (85–204)	n/a	n/a	n/a
Dust (μg/g)						
Range	38–99	17–959	—	—	—	—
Median (IQR)	77 (55–95)	314 (143–543)				
Paint (μg/g)						
Range	—	—	12–154	12–235	1	9
Median (IQR)			33 (17–99)	n/a	n/a	n/a
(b) *Lead in the environment of the schools*						
n (schools)	11	10	4	2	1	1
Water (μg/L)						
Range	0–0.5	0.7–3.5	0–0.2	0.2–1	1.2	0.2
Median (IQR)	0 (0–0.1)	1.9 (1.4–2.1)	0 (0–0.1)	n/a	n/a	n/a
Soil (μg/g)						
Range	1.4–25.6	117–904	5.1–231	2.8–3.9	35.2	3.4
Median (IQR)	9.9 (3.7–14.6)	436 (178–731)	17.6 (5.1–129)	n/a	n/a	n/a
Dust (μg/g)						
Range	11–252	10.7–767	—	—	—	—
Median (IQR)	115 (80–166)	114 (31–211)				
Paint (μg/g)						
Range	70–4728	1.4–8653	119–1883	4618–23221	2067	88
Median (IQR)	749 (320–1866)	56 (5–2602)	1171 (335–1789)	n/a	n/a	n/a

IQR, inter-quartile range.
Note that certain measurements were not made in some centres e.g. Mn and Pb in dust in Kimberley.

quadratic effect, whereby the rate of increase of ln(Mn) with increasing blood lead level decreases at increasing lead levels; for the three sites in the Orange River Valley there was no evidence of a quadratic effect, but there was a significant linear effect of the same magnitude as that found in Cape Town.

The fact that the effect of blood lead on ln(Mn) was not consistent over the four sites and in addition to the fact that the four sites were purposively chosen would lead us to be very cautious about claiming that there is in fact an overall association between ln(Mn) and blood lead level. A graph of the fitted relationship and the observed ln(Mn) values by centre shows the large variability in ln(Mn) in each centre compared to the small trend with increasing lead level (see Fig. 1).

Mixed models were then fitted with the blood lead level as the response variable, the blood manganese variable as the principal explanatory factor, a factor for centre (as before), the same potential confounders as for the models for ln(Mn) and school as a random effect. Again terms were included for blood manganese level and manganese level squared in order to allow for a relationship. The results from this modelling are shown in Table 4(a). The important confounders were found to be gender (with blood lead level being lower in females), race (with blood lead level being lowest for Indians and Whites and highest for coloureds), paint peeling indoors (which led to higher lead levels), and child's pica (which leads to higher levels). Adjusting for these there was strong evidence of a linear effect of blood manganese level (with lead level increasing with increasing manganese level) and some evidence of a negative quadratic effect (i.e. at higher manganese level the line starts to curve downwards).

As for ln(Mn) a test was then carried out to see whether the linear and quadratic effects could be regarded as being homogeneous over the four centres. As was the case for ln(Mn), there was overwhelming evidence that the relationship between blood lead level and blood manganese level was not homogeneous (see Table 4(b)). To examine this heterogeneity further separate models were fitted to each centre with the following results, Cape Town: there was a significant linear trend with a slope of 0.26 and some evidence of a quadratic effect; Johannesburg—there was no evidence of a quadratic effect and the linear trend of 0.011 was not significant; Kimberley—there was no evidence of a quadratic effect and the linear trend of 0.040 was not significant and the Orange River Valley there was no evidence for a quadratic effect, but the linear trend of 0.20 was significant. Fig. 2 shows that the variability in blood lead levels is large compared to the small trend with increasing manganese levels.

Table 3
Results of fitting mixed model to $\log_e$(Mn)

Parameter	Overall	Cape Town	Johannesburg	Kimberley	Orange River Values
(a) *Parameter estimates*					
Constant	1.61 (1.46; 1.76)	1.55 (1.39; 1.71)	2.13 (1.98; 2.28)	1.92 (1.72; 2.12)	1.76 (1.11; 2.40)
Lead	0.021 (−0.002; 0.045)	0.025 (0.010; 0.039)	0.0016 (−0.010; 0.013)	0.045 (0.003; 0.087)	0.023 (0.0010; 0.046)
Lead squared	−0.00044 (−0.0017; 0.0008)	*	*	−0.0019 (−0.004; 0.0002)	*
Female vs. male	0.068 (0.028; 0.11)	0.076 (−0.005; 0.16)	0.023 (−0.045; 0.092)	0.10 (0.03; 0.17)	0.051 (−0.06; 0.16)
Race Coloured vs. African Black	0.084 (0.032; 0.14)	0.091 (−0.016; 0.20)	0.099 (0.008; 0.19)	0.042 (−0.03; 0.11)	0.140 (−0.47; 0.75)
Race other vs. African Black	0.063 (−0.08; 0.21)	0.22 (−0.006; 0.45)	−0.04 (−0.33; 0.25)	−0.25 (−0.54; 0.05)	0.31 (−0.40; 1.01)
Paint peeling from outside Walls	0.040 (−0.004; 0.084)	0.029 (−0.062; 0.12)	0.045 (−0.033; 0.12)	0.049 (−0.03; 0.13)	0.020 (−0.10; 0.14)

(b) *Wald tests for fixed effects*
Dropping individual terms from full fixed model

Fixed term	Wald statistic	df	Wald/df	Chi-square probability
Lead square × centre	24.48	3	8.16	<0.0001
Lead × centre	29.82	3	9.94	<0.0001
Gender	11.32	1	11.32	0.0008
Race	8.24	2	4.12	0.016
Paint peeling from outside wall	2.34	1	2.34	0.126

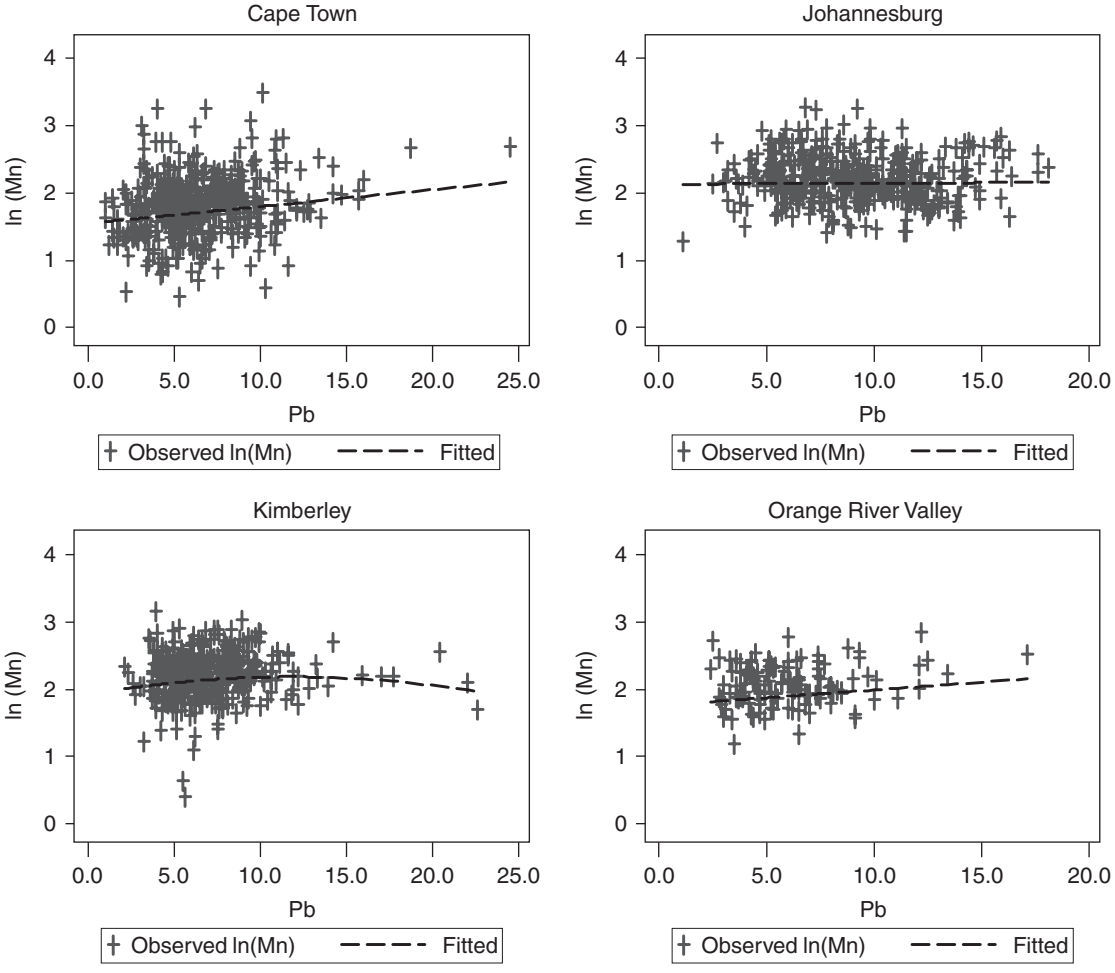

Fig. 1. Log manganese level vs. lead level by centre.

Table 4
Results of fitting mixed models to Pb

Parameter	Overall	Cape Town	Johannesburg	Kimberley	Orange River Valley
(a) *Parameter estimates*					
Constant	5.85 (4.89; 6.82)	5.03 (3.79; 6.27)	9.75 (8.66; 10.84)	6.96 (5.86; 8.07)	7.08 (1.94; 12.22)
Manganese	0.21 (0.057; 0.36)	0.26 (0.054; 0.46)	0.011 (−0.077; 0.098)	0.040 (−0.053; 0.13)	0.20 (0.016; 0.38)
Manganese squared	−0.0059 (−0.012; 0.0003)	−0.0057 (−0.014; 0.0022)	*	*	*
Female vs. male	−0.50 (−0.81; −0.19)	−0.54 (−1.07; −0.019)	−1.14 (−1.73; −0.55)	0.18 (−0.40; 0.76)	0.087 (−0.86; 1.03)
Race Coloured vs. African Black	0.65 (0.26; 1.05)	1.09 (0.41; 1.76)	0.27 (−0.52; 1.06)	0.60 (0.018; 1.17)	−2.30 (−7.40; 2.79)
Race other vs. African Black	−1.47 (−2.55; −0.39)	−1.96 (−3.36; −0.56)	−0.88 (−3.69; 1.94)	−0.15 (−2.57; 2.27)	−4.39 (−10.20; 1.42)
Paint peeling from inside walls	−0.60 (−0.93; −0.26)	−0.10 (−0.68; 0.47)	−0.80 (−1.41; −0.18)	−0.98 (−1.64; −0.31)	−0.50 (−1.55; 0.55)
Child putting paint in mouth	1.31 (0.42; 2.19)	−0.55 (−2.17; 1.06)	2.62 (0.91; 4.34)	2.25 (0.69; 3.81)	−0.89 (−3.44; 1.66)

(b) *Wald tests for fixed effects*
Dropping individual terms from full fixed model

Fixed term	Wald statistic	df	Wald/df	Chi-square probability
Manganese square × centre	16.08	3	5.36	0.0011
Manganese × centre	28.34	3	9.45	<0.0001
Gender	9.70	1	9.70	0.0018
Race	23.06	2	11.53	<0.0001
Paint peeling from inside walls	11.83	1	11.83	0.0006
Child putting paint in mouth	8.68	1	8.68	0.0032

4. Discussion

Chronic exposure to metals, such as manganese, lead, iron and chromium, has been linked to the development of severe, often irreversible, neurological disorders (Roth and Garrick, 2003). The competition for binding ligands between metal ions with similar chemical and physical properties may change a dynamic steady state of equilibrium. Initially, small changes will not affect the organism and might even help to produce adaptability but significant changes will interfere with the functions of different ligands and result in metabolic changes. Similarly, competitive interaction between non-essential metal ions and essential or beneficial metal ions will also interfere with functions of many ligands and produce severe metabolic disturbances. These disturbances depend on the type as well as on the dose of the metal ion concerned as well as on the mode of exposure (Gulumian et al., 1995).

Both lead and manganese are able to cross the blood-brain barrier and deposit in the brain, although their mechanisms of initiating CNS cell loss might be different. It is also possible that cellular uptake of manganese may be influenced by the concentration of another divalent toxic metal such as lead and iron (Roth and Garrick, 2003; Erikson et al., 2002).

Similarly, in an anticipation of the increase in use of MMT in South African fuels starting at the beginning of year 2006, when only unleaded fuels will be available to motorists, our study investigated the possible association between levels of these two metals in bloods of schoolchildren (mean age 7 years) residing in different geographical regions of the country. Study found that the levels of manganese in blood were significantly lower in Cape Town when compared with Johannesburg (where MMT was introduced to petrol 24 months before survey) and other sites. Furthermore, blood lead concentration was lowest in Orange River Valley, followed by Cape Town and highest in Johannesburg. In both cases the differences between four centres are highly significant with $P<0.0001$ for each metal. It was also found that blood lead levels in Johannesburg decreased on average by 2.8 μg/dL since the introduction of unleaded petrol, used by approximately 30% of vehicles.

When fitting mixed models and adjusting for confounders, the relationship between log(Mn) and blood lead level was not consistent over four sites. Since the four sites were purposely selected, and since there was no evidence of a relationship in Johannesburg, which had the highest levels of both manganese and lead in blood, we should be very cautious about concluding that there is an overall association between blood manganese and lead in schoolchildren. More work needs to be done to ascertain the effect of introducing MMT in developing countries where socio economic and health characteristics of population differ from those in developed countries.

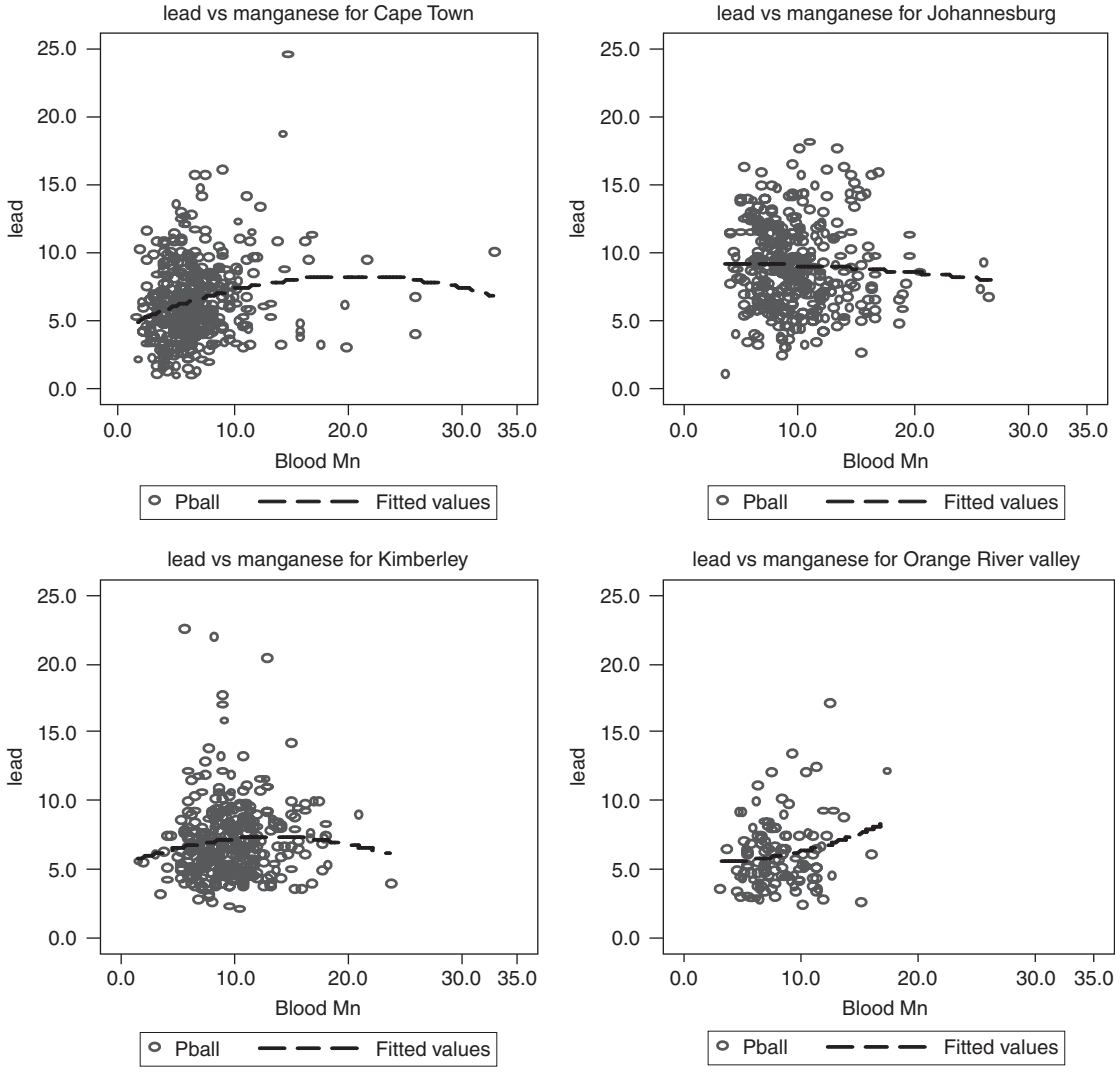

Fig. 2. Lead level vs. manganese level by centre.

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Ethical considerations: The study protocol was submitted to the Committee for Research on Human Subjects of the University of the Witwatersrand and ethical approval was obtained (Clearance Certificate Number M01-05-16). Informed written consent from parents/guardians of every child was obtained before commencement of the study.

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